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EXAMINER	
PANDE, SUCHIRA	

ART UNIT	PAPER NUMBER
1637	

DATE MAILED: 11/09/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/527,618	AU-YEUNG ET AL.	
	Examiner	Art Unit	
	Suchira Pande	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *11 October 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *16-32* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☒ Claim(s) *32* is/are allowed.

6)☒ Claim(s) *16-28 and 31* is/are rejected.

7)☒ Claim(s) *29 and 30* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *11 March 2005* is/are: a)☐ accepted or b)☒ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)

2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)

3) ☒ Information Disclosure Statement(s) (PTO/SB/08)
Paper No(s)/Mail Date *4/4/05,12/19/05*.

4) ☐ Interview Summary (PTO-413)
Paper No(s)/Mail Date. _____ .

5) ☐ Notice of Informal Patent Application

6) ☐ Other: _____.

DETAILED ACTION

Election/Restrictions

1. Applicant's election without traverse of group II invention claims 16-23 in the reply filed on October 11, 2006 is acknowledged. Applicant cancelled claims 1-15 and added new claims 24-32. Currently claims 16-32 are pending in the application.

2. Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 CFR 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a request under 37 CFR 1.48(b) and by the fee required under 37 CFR 1.17(i).

Drawings

3. The drawings are objected to because casing 32 housing sparge stone 31 is not clear in Fig. 3. Corrected drawing sheets in compliance with 37 CFR 1.121(d) are required in reply to the Office action to avoid abandonment of the application. Any amended replacement drawing sheet should include all of the figures appearing on the immediate prior version of the sheet, even if only one figure is being amended. The figure or figure number of an amended drawing should not be labeled as "amended." If a drawing figure is to be canceled, the appropriate figure must be removed from the replacement sheet, and where necessary, the remaining figures must be renumbered and appropriate changes made to the brief description of the several views of the drawings for consistency. Additional replacement sheets may be necessary to show the

renumbering of the remaining figures. Each drawing sheet submitted after the filing date of an application must be labeled in the top margin as either "Replacement Sheet" or "New Sheet" pursuant to 37 CFR 1.121(d). If the changes are not accepted by the examiner, the applicant will be notified and informed of any required corrective action in the next Office action. The objection to the drawings will not be held in abeyance.

Claim Rejections - 35 USC § 102

4. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

5. Claims 20 and 21 are rejected under 35 U.S.C. 102(b) as being anticipated by Ciccolini et. al. (1999) Bioprocess Engineering 21: pp 231-237 and evidenced by Wan et. al. (US Pat. 5,837,529 issued November 17, 1998)

Regarding claim 20, Ciccolini et. al. teaches a cell lysis process wherein cells in suspension are lysed in the presence of a controlled stream of gas bubbles sufficient to cause flotation and separation of a cellular debris component over a clarified lysate component comprising extrachromosomal nucleic acids. (see page 231, par. 3-page 232 par. 1 where cell lysis and floatation of cell debris is taught). On page 236 bottom of par. 5, Ciccolini et. al. teaches mixing using "two-impinging –jet' mixing and on page page 237 par. 1 teaches mixing of injected potassium acetate and air and separation of the flocculated material from plasmid containing liquor. The air is in form of small

bubbles (controlled stream of gas bubbles). The fact that the cellular debris formed by their method is flocculant material that floats inherently indicates that the controlled stream of air that is mixed creates a controlled stream of gas bubbles is sufficient to cause flotation and separation.

Regarding claim 21, Ciccolini et. al. further teaches wherein the lysis process is an in-line process for alkaline lysis of bacterial cells (see page 236, par. 5) as evidenced by Wan et. al. who describe in detail the in-line process for alkaline lysis see whole patent, specially see col. 3 where in-line lysis is taught and col. 3, lines 20-21 where alkali is taught as a lysis agent)

Claim Rejections - 35 USC § 103

6. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

7. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to

consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

8. Claims 16- 19 are rejected under 35 U.S.C. 103(a) as being unpatentable over Cuthbertson (WO 99/55837 published November 4, 1999) in view of Theodossiou et. al. (1999) Bioprocess Eng. 20: 147-156.

A) Regarding claim 16, Cuthbertson teaches A process of clarifying a bacterial lysate comprising plasmid DNA and cellular debris (see page 12, lines 1-5 where fragments of bacterial cells, cell organelles, DNA, RNA etc are taught) comprising the steps of:

(a) introducing a gas into a fluid stream under conditions forming an entrainment of bubbles in the fluid stream (See page 4, lines 34-35 where use of free gas bubbles or micorbubbles is taught for floatation separation. Also see page 9, lines 9-15 where use of different gases to produce microbubbles is taught);

(b) wherein the entrained bubbles generate a buoyant precipitate comprising the cellular debris (see page 11, lines 1-29 where flotation of microbubble/target samples is taught.);

(c) allowing the buoyant precipitate to coalesce and separate over a fluid phase (See page 11, lines 26-29 where broad teaching is provided that the process of the invention may in principle be used to separate any target component which is suitable for separation from a liquid sample using floatable microbubbles); and

(d) collecting the fluid phase (see abstract where negative separation process is taught. Here recovery of target-free sample (liquid phase containing) material is taught following separation of the floating layer.

B) Regarding claim 16, Cuthbertson does not teach:

1) a suspended bacterial cell suspension and a lysis buffer

2) admixing a precipitation buffer into the fluid stream,

3) a fluid phase comprising the plasmid DNA

4) collecting the fluid phase comprising the plasmid DNA

Regarding claim 18, Cuthbertson teaches wherein the gas is introduced through an aperture comprising a plurality of pores of less than approximately 5 microns in diameter. (see page 11, line 9 where microbubbles in size range of 3 to 5 microns (μm) in diameter are taught.)

Regarding claim 19, Cuthbertson teaches wherein the pores are approximately 2 microns in diameter (see page 11, line 9 where microbubbles in size range of 1 to 10 microns (μm) in diameter are taught. Thereby teaching microbubbles of approximately 2 microns in diameter).

Regarding claims 18 and 19 it should be noted that Cuthbertson does not specify the size of the aperture through which gas is introduced but the fact that he teaches bubbles of the right size diameter inherently implies that the gas is being introduced through aperture of appropriate sizes.

C) Regarding claim 16, Theodossiou et. al. teaches:

A process of clarifying a bacterial lysate comprising plasmid DNA and cellular debris, comprising the steps of:

(a) a suspended bacterial cell suspension and a lysis (see page 148, section 2.2 par. 2 where bacterial cells resuspended in a suspension buffer and an alkaline lysis buffer are taught)

(b) admixing a precipitation buffer, wherein a buoyant precipitate comprising the cellular debris is formed (see page 148, section 2.2 par. 2, where addition of precipitation buffer potassium acetate is taught and page 152 section 3.2 where flotation of cell debris is taught);

(c) allowing the buoyant precipitate to coalesce and separate over a fluid phase comprising the plasmid DNA (see page 152 section 3.2 par. 1 where formation of off-white gelatinous solids that float upwards are taught and fluid phase 'cleared' plasmid containing is below this buoyant solid precipitate) ; and

(d) collecting the fluid phase comprising the plasmid DNA (see page 152 section 3.2 par. 1 where collection of plasmid containing liquid from beneath the floating floc by draining is taught).

Regarding claim 17, Theodossiou et. al. teaches wherein the lysis buffer is an alkaline lysis buffer (see page 148, section 2.2 par. 2 where an alkaline lysis buffer is taught).

D) It would have been *prima facie* obvious to one of ordinary skill in the art to practice the method of Theodossiou et. al. in the method of Cuthbertson at the time of

this invention to clarify bacterial lysates for isolating plasmids. The motivation to do so is provided both by Theodossiou et. al.

Theodossiou et. al. state "The efficiency of flotation at 15 l scale with respect to 'clearing' of the liquor and formation of a tight floating layer of solids at the air-liquid interface appears to be assisted by entrapment of air within the solids.------The efficiency of flotation is inextricably linked to mixing and clearly to make this approach scalable both upward and downward will require precise control of the mixing conditions" (see page 153 par. 1).

By combining the method of Theodossiou et. al. in the method of Cuthbertson one of ordinary skill in the art arrives at a method of plasmid isolation that is both scalable and allows rapid and gentle mixing (low shear) of the different solutions. Use of air to form bubbles performs tow fold function 1) it allows for rapid and gentle mixing and 2) improves efficiency of flotation of cellular debris..

Air bubbles can be used to improve both quality and quantity of plasmids useful for pharmaceutical purposes is evidenced by Levy et. al. (2000) Trends in Biotechnol. 18:296-305. See whole article specially see page 300 bottom of par. 4 where Levy et. al. describe the use of additional air sparging at base of reactor and state "additional air sparged at the base of the reactor provides 'low –shear' mixing throughout the neutralization that further enhances the up-flow of flocs to the surface"

9. Claim 22 is rejected under 35 U.S.C. 103(a) as being unpatentable over Chevalier (WO 99/37750 published on July 29, 1999). US equivalent of the above WO

document (US Pat. No 6,664,049 is being used to point out pertinent sections as it is easier to cite US patents) in view of Ciccolini et. al. (1999) Bioprocess Engineering 21: pp 231-237 and Mittlestaedt et. al. (US Pat. 6, 268,492 issued July 31, 2001) as evidenced by Theodossiou et. al. (1999) Bioprocess Eng. 20: 147-156.

Regarding claim 22, Chevalier teaches: a method for the purification of extrachromosomal DNA from a pharmaceutical grade bacterial fermentation (see col. 2. lines 49-50 and lines 57-60), comprising the steps of:

(a) generating a fluidized stream of bacterial cells (see col. 4. lines 55-56 where stream of cell suspension is taught);

(b) introducing a lysis buffer to form a cell lysate solution (see col. 4. lines 57-60);

(c) introducing a precipitation solution into the cell lysate solution (see col. 4 lines 61-64);

Regarding claim 22, Chevalier does not teach:

(b) introducing a gas into the fluidized stream to form a solution comprising a plurality of bubbles;

(c) introducing a precipitation solution into the cell lysate solution wherein combined action of the bubbles and the precipitation solution results in the formation of a buoyant precipitate comprising cell debris and chromosomal DNA;

(d) allowing the buoyant precipitate to coalesce and separate from an underlying fluid phase comprising the extrachromosomal DNA;

(e) collecting underlying fluid phase to form a clarified lysate;

(f) filtering the clarified lysate;

and (g) subjecting the filtered clarified lysate to ion exchange chromatography to separate the extrachromosomal DNA from residual contaminants.

Regarding claim 22, Ciccolini et. al. teaches:

(b) introducing a gas into the fluidized stream to form a solution comprising a plurality of bubbles (see page 237, par. 1 where presence of introduced air in the form of small and its advantages are taught);

(c) introducing a precipitation solution into the cell lysate solution wherein combined action of the bubbles and the precipitation solution results in the formation of a buoyant precipitate comprising cell debris and chromosomal DNA (see page 237, par. 1 where potassium acetate (precipitation solution) injection along with air is taught. The presence of small air bubbles allows for low shear mixing thus facilitating the formation of a buoyant precipitate (flocculated material) comprising cell debris and chromosomal DNA ;

(d) allowing the buoyant precipitate to coalesce and separate from an underlying fluid phase comprising the extrachromosomal DNA (see page 237, par. 1 where separation of underlying plasmid (extrachromosomal DNA) containing liquor from coalesced buoyant (low density of flocculated material) precipitate is taught;

e) collecting underlying fluid phase to form a clarified lysate (see page 237, par. 1 Ciccolini et. al. does not provide details of how this extrachromosomal DNA is further purified. They say the plasmid containing liquor was processed as described before. The reference provided is Theodossiou et. al. (1999) Bioprocess Eng. 20: 147-156).

Theodossiou et. al. on page 152 par.1 teach recovering 'cleared' plasmid containing liquid from underneath the floating floc by draining the clarified lysate.

(f) filtering the clarified lysate (Theodossiou et. al. on page 153 also see Table 5 teach filtering the clarified lysate through many different filtration materials ; and

(g) subjecting the filtered clarified lysate to ion exchange chromatography to separate the extrachromosomal DNA from residual contaminants (see page 155 end of par.1 conclusion where use of packed bed chromatographic columns is taught).

Regarding claim 22, Ciccolini et. al. as evidenced by Theodossiou et. al. fails to specifically teach use of ion exchange chromatography to separate the extrachromosomal DNA from residual contaminants

Regarding claim 22, Mittlestaedt et. al. teaches:

f) filtering the clarified lysate (see fig. 1 where a depth filter is used to filter the lysate and see col. 4 lines 34-62); and

(g) subjecting the filtered clarified lysate to ion exchange chromatography to separate the extrachromosomal DNA from residual contaminants (see col. 5, lines 4-5).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to combine the method of Ciccolini et. al. as evidenced by Theodossiou et. al. in the method of Chevalier. The motivation to do so is provided by both Chevalier and Theodossiou et. al.

Chevalier points out the disadvantages associated with manual alkaline lysis technique of Birnboim used for lysing bacterial cells for industrial scale plasmid preparations (see col. 1, lines 36-67). He points out importance of proper mixing. "An

insufficient stirring results in a lysis of poor quality whereas an excessive stirring tends to fragment the genomic DNA, which subsequently mixes with the plasmids. In both cases, a reduction in the plasmid DNA yield is observed. Consequently, the dexterity of the operator is an essential requirement for the success of the operation". He goes on to describe various methods proposed to automate the method of lysis and their disadvantages (see col. 2 lines 1-30). Finally he states "The present invention is intended to overcome these disadvantages and to provide a method of cell lysis which is applicable in particular to the extraction and purification of nucleic acids such as plasmids from bacteria -------and which is capable of being used without manual intervention (in-line process) ----------. Another objective is to provide on the industrial scale plasmid preparations with a high and substantially enhanced yield compared to that for preparations which may be obtained according to the prior art methods" (see col. 2 lines 33-60)

Theodossiou et. al. on page 155 par. 1 state "Clearly separation of the soild cellular debris from the plasmid containing liquor by floatation is a simple and promising approach.------Though a thorough understanding of the mechanism of floc formation and its flotation properties may make it possible to deliver a 'cleared' plasmid liquor of exceptional clarity combined with high recovery, further polishing prior to application to packed bed chromatographic columns will undoubtedly be required". Thus by practicing the method of Ciccolini et. al. in the method of Chevalier one of ordinary skill in the art gets the benefit of both a process suitable for pharmaceutical scale processing and get

plasmid liquor of exceptional clarity due to use of floating flocs formed by use of small air bubbles as taught by Ciccolini et. al.

Theodossiou et. al. also provides the suggestion to one of ordinary skill in the art for use of chromatographic columns. Mittlestaedt et. al. provide the motivation to combine the use of ion exchange columns in the method of Chevalier and Ciccolini et. al. Mittlestaedt et. al. state "while many methods exist for purification of nucleic acid molecules, such methods are often limited when the nucleic acid molecules are to be produced for therapeutic purposes, since they must be prepared free of any contaminants such as toxic compounds and antigenic molecules.------. The present invention provides methods for producing purified nucleic acid molecules that are suitable for use in pharmaceutical applications, and on a large scale." (see col. 1, lines 34-57).

10. Claims 23, 28 and 31 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wan et. al. (US Pat. 5,837,529 issued November 17, 1998) in view of Cuthbertson (WO 99/55837 published November 4, 1999).

Regarding claim 23, Wan et al. teaches: a method of producing a clarified cell lysate comprising plasmid DNA from an alkaline bacterial cell lysate (see title and abstract), comprising the steps of:

introducing a suspension of bacterial cells into a fluid flow comprising an alkaline lysis buffer (see Fig. 3 where bacterial cell suspension from mixer 1 is introduced into fluid flow)

wherein the cells are flowably mixed with the cell lysis buffer thereby forming a cell lysis mixture (see fig. 3 where cells are flowably mixed with cell lysing solution in mixer 2 thereby forming cell lysis mixture);

introducing a precipitation buffer into the fluid flow comprising the cell lysis mixture, thereby forming a cell debris precipitate in the cell lysis mixture (see fig. 3 where precipitation solution is introduced into mixer 3 and precipitate of cell debris is formed) ;

Regarding claim 23, Wan et. al. do not teach :

an entrainment of gas, wherein the cells are flowably mixed with the cell lysis buffer together with the gas thereby forming a cell lysis mixture;

separating the mixture into a buoyant flocculent phase comprising the precipitated cell debris and a fluid phase comprising a substantially clarified cell lysate; and isolating the substantially clarified cell lysate.

Regarding claim 28, Wan et. al. teaches static mixer (see abstract and figs 1 and 2)

Regarding claim 31, Wan et. al. teaches a contained continuous flow fluid path (see Fig. 1, 2 and 3)

Regarding claim 23, Cuthbertson teaches:

an entrainment of gas (See Cuthbertson page 4, lines 34-35 where use of free gas bubbles or micorbubbles is taught for floatation. Also see page 9, lines 9-15 where use of different gases to produce microbubbles is taught). If the gas is introduced into

the cell lysis buffer then we arrive at the situation wherein the cells are flowably mixed with the cell lysis buffer together with the gas thereby forming a cell lysis mixture.

separating the mixture into a buoyant flocculent phase comprising the precipitated cell debris and a fluid phase comprising a substantially clarified cell lysate; and isolating the substantially clarified cell lysate.(See Cuthbertson abstract where negative separation process of removing buoyant flocculent phase from liquid is taught).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to combine the method of using gas entrainment taught by Cuthbertson in the method of alkaline lysis taught by Wan et. al. The motivation to do so is provided by Ciccolini et. al. who state in page 236 end of last par. "what is needed is a process with the capacity to provide mixing times of the order of a second, after which the mixing intensity must be reduced to a minimum in order to avoid fragmenting the chromosomal DNA. Additionally, the neutralization step requires a degree of mixing but at low shear in order to minimize damage to the fragile flocculated material". Thus addition of gas to form air bubbles to lysis solution would allow for rapid mixing in the method of Wan et. al. that would be gentle thereby minimizing the fragmentation of denatured cell debris. Further the presence of air bubbles will aid the flocculated cell debris that naturally has a low density to stay afloat thereby aiding in formation of substantially clarified cell lysate meaning the plasmid containing liquor isolated from this process will be substantially free of cell debris.

11. Claims 24-27 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wan et. al. (US Pat. 5,837,529 issued November 17, 1998) in view of Cuthbertson (WO 99/55837 published November 4, 1999) as applied to claim 23 above further in view of Gomez et. al. (2000) Canadian J. of Chemical Engineering Sciences vol. 78 (4) 785-792.

Regarding claim 24, Wan et. al. and Cuthbertson teach the method of producing an alkaline bacterial cell lysate of claim 23. But Wan et. al. and Cuthbertson do not teach wherein the gas is introduced via a gas port through which gas is forced under pressure into the fluid flow thereby controllably forming bubbles in the cell lysis mixture.

Regarding claim 24, Gomez et. al. teaches wherein the gas (air) is introduced via a gas port through which gas is forced under pressure into the fluid flow thereby controllably forming bubbles in the mixture (see page 788 fig. 2 where air is forced to enter the column through bottom (gas port). The air is introduced into the fluid flow via vertical sparger. The air bubbles formed are controlled such that measurement of their size etc. is done at the top of the column (see page 788 section on Experimental design for details).

Regarding claim 25, Gomez et. al. teaches wherein the gas port comprises an aperture comprising a plurality of pores (see page 788 par. 3 under experimental design where Gomes teaches a sparger made from a piece of porous (comprising a plurality of pores) SS tube. Thereby Gomez et. al. teaches the gas port comprises an aperture comprising a plurality of pores.

Regarding claim 26, Gomez et. al. teaches wherein the pores have an average diameter of less than approximately 5 microns. (see page 791 Figure 7, where sparger with a pore diameter of 2 μM and 3 μM is taught). Thereby Gomez et. al. teaches wherein the pores have an average diameter of less than approximately 5 microns.

Regarding claim 27, Gomez et. al. teaches wherein the aperture comprising a plurality of pores is a sparger comprising pores having an approximate average diameter of 2 microns or less (see page 789 par. 4 where nominal pore diameter of 1μM designated by manufacturer of sparge stone and estimated pore diameter of 2μM based on actual bubble size measurement is taught). Thereby Gomez et. al. teaches wherein the aperture comprising a plurality of pores is a sparger comprising pores having an approximate average diameter of 2 microns or less.

It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to combine the method of using sparger with the pores of 2 μM or less taught by Gomez et. al. in the method of alkaline lysis taught by Wan et. al. and Cuthbertson. The motivation to do so is provided by Kawagoe et. al. (1975) J. of Chemical Engineering of Japan vol. 8 (3) 254-6. Kawagoe et. al. teaches the effect of gas liquid flow pattern on bubble size. They teach gas liquid flow pattern can be classified into three typical regions that are bubble flow, froth and cellular foam regions. This in turn depends on gas velocity (see page 254 par. 7). In light of teaching so Kawagoe et. al. it would be obvious to one of ordinary skill in the art that since the object of the invention is to float the coalesced alkaline cell lysis debris by using sparged gas air bubbles the velocity with which the gas is sparged into the system is

important. The velocity of bubbles produced depends on the size and type of gas port through which the gas is introduced into the liquid. It is well known in the art that for air-water system, 94% of the gas kinetic energy is dissipated very close to the sparger exit. The remaining 6% is dissipated at distances greater than 18 sparger diameters (Sridhar et. al. 1980 Industrial & Engineering Chemistry Fundamentals vol. 19 (1) pp 21-6). Hence indicating why size of the diameter of the pores in the sparger has to be of a specific size range.

Allowable Subject Matter

12. Regarding claims 29-30 prior art does not teach a method of plasmid production where pH adjustment buffer is added to the precipitated lysate followed by separation of flocculant phase in a lysate separation tank.

13. Claim 32 is allowable. Prior art does not teach following elements of the claim

a) introducing a pH adjustment buffer into the fluid flow comprising cell debris precipitate. Prior art teaches pH adjustment at the time of neutralization by addition of the precipitation solution which results in formation of the cell debris precipitate but not after the formation of precipitate.

b) flowing the pH adjusted cell lysis mixture into a lysate separation tank.

Conclusion

14. Claims 16-28 and 31 are rejected over prior art.

15. Claims 29-30 are objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims as they are free of prior art.

16. Claim 32 is allowable.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Suchira Pande whose telephone number is 571-272-9052. The examiner can normally be reached on 8:30 am -5:00 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on 571-272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

JEFFREY FREDMAN
PRIMARY EXAMINER
11/21/06

Suchira Pande
Examiner
Art Unit 1637